Radiation Protection Dosimetry (2015), pp. 1–4 doi:10.1093/rpd/ncv169

IMPACT OF INTERCELLULAR INDUCTION OF APOPTOSIS ON LOW-DOSE RADIATION CARCINOGENESIS

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In vitro **data indicate that selective removal of oncogenic transformed cells by apoptosis induced via signalling by neighbouring cells may represent an important anti-carcinogenic process. Mechanistic modelling supports this concept and predicts that the phenomenon can stop the growth of a transformed cell population, forming a dormant pre-neoplastic lesion, or even remove the transformed clone completely. Radiation has been shown to enhance the underpinning signalling and increase the extent and rate of apoptosis induction in precancerous cells. Implications for low-dose radiation carcinogenesis are discussed based on *in vitro* data and mechanistic modelling. The possibility is outlined for radiation to act in a pro-carcinogenic manner, i.e. to reduce rather than enhance the removal of transformed cells by apoptosis. The effects of radiation exposure during early or late carcinogenesis are discussed.**

INTRODUCTION

Oncogenic transformed cells represent an *in vitro* system that mimics early-stage carcinogenesis (Figure 1). They possess some of the hallmarks of cancer, such as self-sufficiency in growth signals or insensitivity to anti-growth signals[1, 2]. They induce tumours upon injection into animals but differ from the later extracted tumour cells[3]. Transformed cells thus 'reflect the cell culture equivalent of initiation'[4].

When co-cultured with normal cells, transformed cells are selectively removed by apoptosis[5, 6]. This intercellular induction of apoptosis (IIA) has been suggested to serve as a natural anti-carcinogenic mechanism[5, 6]. The underlying signalling involves cytokines and reactive oxygen and nitrogen species. Low doses of radiation have been shown to enhance the signalling and hence the extent and rate at which transformed cells are removed by IIA[7–9].

In this work, the recently proposed mechanistic model of IIA[10] extended to account for the two major underlying signalling pathways is reviewed. Major implications for radiation-induced modifications of IIA and their relevance to low-dose carcinogenesis are discussed. The possibility is outlined for radiation to affect IIA in a pro-carcinogenic way, i.e. to reduce rather than enhance the removal of transformed cells. The effects of radiation exposure during early or late phases of normal carcinogenesis are discussed.

MECHANISTIC MODELLING OF THE PROCESS

Three distinct steps have been identified in the mechanism leading to the selective removal of oncogenic transformed cells by IIA[6]. First, transformed cells signal via active TGF-β (transforming growth factor type beta). This cytokine triggers the release of nitric oxide and peroxidase from neighbour cells, either normal or also transformed. In the second step, these primary species undergo a cascade of biochemical reactions together with superoxide ($O_2^{-\bullet}$) constitutively released by transformed cells. Two distinct pathways play key roles in IIA[6]. In the peroxidase (POD)/hypochlorous acid (HOCl) pathway, hydrogen peroxide (H_2O_2) formed by dismutation of $O_2^{-\bullet}$ is removed by POD, partially producing HOCl, which upon reaction with $O_2^{-\bullet}$ yields hydroxyl radicals ($^{\bullet}OH$). In the nitric oxide ($NO^{\bullet}$)/peroxynitrite ($ONOO^-$) pathway, rapid reaction between $NO^{\bullet}$ and $O_2^{-\bullet}$ takes place, forming $ONOO^-$, whose decay again produces highly reactive $^{\bullet}OH$. Two less significant IIA pathways[6] are not considered here. Due to the short range of $O_2^{-\bullet}$, in both pathways $^{\bullet}OH$ are produced selectively in the vicinity of $O_2^{-\bullet}$-producing transformed cells. In the third step, $^{\bullet}OH$ radicals attack lipids in cell membranes, which through a complex intracellular cascade triggers the mitochondrial pathway of apoptosis[6].

IIA has been shown active in a large variety of transformed cells challenged by normal or transformed cells of diverse origins, including human cell lines. However, tumour cells are no longer sensitive to IIA, as they express membrane-based catalase that abrogates the underlying signalling[1].

The multi-scale mechanistic model of IIA[10, 11] follows the aforementioned three steps. The TGF-β signalling module may include feedbacks (auto-induction and inhibition) known for this cytokine. In the second module, a set of reaction-diffusion equations depict the intercellular biochemical reaction scheme. Originally developed for the POD/HOCl pathway[10], the model has been recently extended to

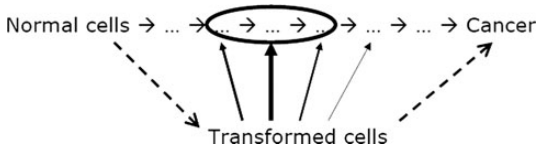

Figure 1. Transformed cells as an *in vitro* model of early-stage carcinogenesis. The *in vitro* transformation steps (left dashed arrow) and the tumour formation following inoculation of transformed cells (right dashed arrow) might, in principle, somewhat differ from the multistage carcinogenesis *in vivo* (upper part of the scheme). If this were the case, transformed cells would be an artificial *in vitro* system of a limited relevance to carcinogenesis. Nevertheless, indirect evidence[1, 4] suggests that transformed cells likely represent an *in vitro* analogue of early-stage carcinogenesis *in vivo* (solid arrows and oval).

the $NO^{\bullet}/ONOO^{-}$ pathway (P. Kundrát *et al.*, in preparation). Finally, the intracellular processes following $^{\bullet}OH$ attacks damaging the membrane are modelled in a phenomenological manner, as a non-linear process triggering apoptosis that competes with cell proliferation[10]. The resulting non-linear, stiff set of partial and ordinary differential equations are solved numerically. Analytical approximations have been developed and benchmarked against numerical simulations (P. Kundrát *et al.*, in preparation).

DORMANT PRE-NEOPLASTIC LESION FROM STOPPED GROWTH OF TRANSFORMED CLONE

The model has been calibrated[10] against *in vitro* data[12] on apoptosis triggered in 208F src3 transformed rat fibroblasts either by well-defined external species ($ONOO^{-}$ donors, H_2O_2 or its donors, enzymatic systems producing H_2O_2, $O_2^{-\bullet}$ or HOCl) or under co-culture with normal 208F cells[13]. As shown in Figure 2, simulations on the HOCl pathway[10] reproduce the data[13] (dashed line and triangles). Also the increased extent and rate of IIA effect by the $NO^{\bullet}$ pathway is modelled correctly (solid line and squares). In some experiments, IIA has killed virtually all transformed cells[14, 15]. Indeed, biphasic IIA kinetics quickly reaching 100 % is predicted for enhanced releases of $NO^{\bullet}$ (dash-dotted line). Similarly, with reduced 'repair' capacity, all cells undergo apoptosis (dotted line).

Based on the *in vitro* data[10, 13] spanning 1–3 d of culture, the model predicts that IIA is capable of stopping the growth of a transformed cell population[10]. This long-term limit on the transformed population growth depends on the release rates of the signalling species, their lifetimes in the medium and cell sensitivities to the inducers. Such a long-term limit is predicted even for '*in vivo*-like' conditions with superoxide lifetime as short as 100 ms estimated in blood[16] and even if the growth of transformed cells *per se* were completely unlimited otherwise[10]. This state of stopped growth likely

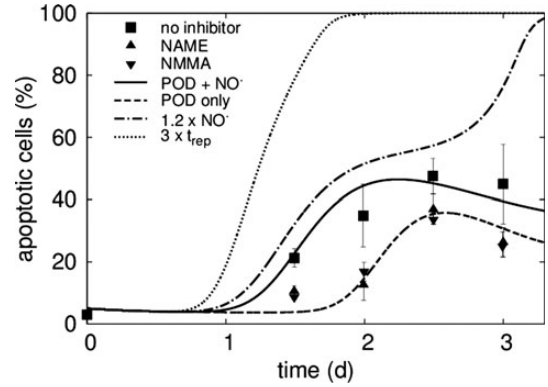

Figure 2. Apoptosis induced in transformed rat fibroblasts upon co-culture with normal fibroblasts. Calculations with peroxidase pathway only[10] (dashed line; 10^{-16} mol s^{-1} of $O_2^{-\bullet}$ released per transformed cell, characteristic repair time of $^{\bullet}OH$-induced damage $t_{rep} = 15$ h) or with both pathways (solid line; $NO^{\bullet}$ lifetime of 3 min, $NO^{\bullet}$ release rates of 2×10^{-19} and 2×10^{-18} mol s^{-1} per transformed and TGF-β pre-treated normal cell, respectively) are compared with data[13] with (filled triangle, filled inverted triangle) and without inhibitors of $NO^{\bullet}$ synthesis (filled square). Predicted effects of 20 % increased $NO^{\bullet}$ release (dash-dotted line) or of reduced cellular capacity to remove the induced damage (dotted line; $t_{rep} = 45$ h) are shown too.

corresponds to a dormant pre-neoplastic lesion[10]. Through stopping the transformed population growth, IIA provides a 'window of opportunity' for the action of other anti-carcinogenic mechanisms, such as the immune system. This window remains open until the phenotypical change of transformed cells to tumour ones, namely until they start expressing catalase that makes the underlying signalling ineffective.

If, as discussed earlier, eventually not just a fraction but all transformed cells were subject to IIA, the phenomenon would still be crucial for the control of carcinogenesis. For a tumour to develop, the mentioned phenotypical change would have to occur before the apoptotic destruction of the transformed population. Carcinogenesis would then be interpreted as a failure of apoptotic signalling, presumably a rather rare event.

RELEASING MORE PRIMARY SIGNALS MAY REDUCE APOPTOSIS INDUCTION

To study IIA systematically, several thousands of simulations have been performed for the co-culture system. Model parameters have been varied such as initial cell densities, release rates and lifetimes of signalling species or cell sensitivities to apoptotic inducers. The results demonstrate distinct non-linear behaviour of the two pathways in different modes. In terms of the resulting $^{\bullet}OH$ flux, the efficiency of the HOCl pathway increases with the second-to-third power of the superoxide release rate (and transformed

cell density), regardless of whether POD originates from transformed or non-transformed cells. The efficiency of the $ONOO^-$ pathway in the autocrine destruction mode, where $NO^\bullet$ comes from transformed cells themselves, increases in a (sub-)linear manner with cell density and $NO^\bullet$ release rate. However, in the inter-culture mode where $NO^\bullet$ is provided by normal cells, the $ONOO^-$ pathway efficiency initially increases about linearly with the amount of $O_2^{-\bullet}$ released but decreases at high $O_2^{-\bullet}$ levels when $NO^\bullet$ becomes limiting (Figure 3).

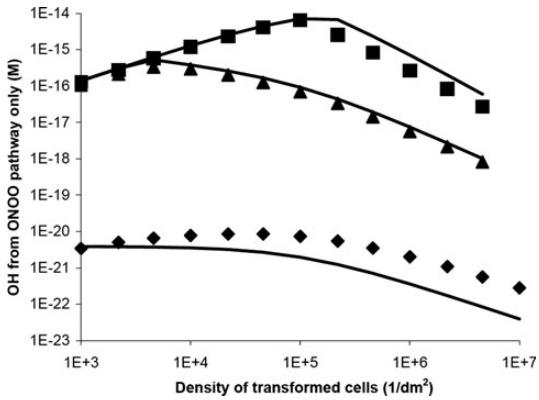

Figure 3. The $ONOO^-$ pathway's efficiency in terms of the resulting $^\bullet OH$ concentrations initially increases about linearly with the density of transformed cells and hence the amount of superoxide produced. When superoxide release is no longer restraining for the diffusion-limited reaction $O_2^{-\bullet} + NO^\bullet \rightarrow ONOO^-$, its further increase tends to reduce the pathway efficiency, as $ONOO^-$ is produced farer away from the transformed cells (P. Kundrát *et al.*, in preparation) and consequently lower $^\bullet OH$ levels are reached at transformed cells. Points: numerical simulations, lines: analytical approximations (P. Kundrát *et al.*, in preparation), with $O_2^{-\bullet}$ and $NO^\bullet$ release rates of 10^{-16} mol s^{-1} per transformed and non-transformed cell, respectively, density of transformed cells as indicated, non-transformed cells 10^6 dm^{-2}, $O_2^{-\bullet}$ lifetime 1.7 s and $NO^\bullet$ lifetimes of 1 s (filled diamond), 10 s (filled triangle) and 100 s (filled square), respectively.

When both pathways are effective, the resulting kinetics of IIA may exhibit complex behaviour (P. Kundrát *et al.*, in preparation) including biphasic patterns, reach 100 % or stay well below this value, as seen in the experiments[1, 13, 14]. The behaviour depends on system parameters governing the signalling magnitude, cell sensitivity and ability to remove the signalling effects (cf. Figure 2).

RELEVANCE TO LOW-DOSE CARCINOGENESIS

Low-dose radiation modifies the underlying signalling and hence the extent and rate of IIA[7]. Enhanced IIA has been reported after irradiating normal cells already with 2 mGy of ^{60}Co gamma rays or 0.3 mGy of 3 MeV alpha particles; the effect increases with dose, saturates at 25–50 mGy, but remains enhanced till the highest dose applied, 2 Gy[7, 14, 15]. An 8-fold increase in $O_2^{-\bullet}$ release by transformed cells and 2- to 4-fold enhancement of POD levels have been observed following 20–200 mGy of ^{137}Cs gamma irradiation[8, 9]. These effects are mediated through modified TGF-β signalling and are transient only[8]. Unfortunately, sufficient data are not available on the amount and kinetics of TGF-β signalling by transformed cells and its perturbation by low-dose radiation. Data directly linking TGF-β levels with release rates of primary species (POD and/or $NO^\bullet$) are lacking, either. Thus, the related modelling will likely have to infer the release rates indirectly, by comparing the calculated kinetics of apoptosis with the corresponding data.

However, already the present model and the existing experiments do provide valuable insights into the relevance of IIA to low-dose carcinogenesis. Typically, enhanced release of signalling species ($O_2^{-\bullet}$ by irradiated transformed cells, or POD and/or $NO^\bullet$ by irradiated non-transformed or transformed cells) tends to increase the efficiency of the signalling and, hence, the extent and/or rate of IIA. This has been observed in a number of experiments[7, 8, 14]. The enhanced removal of transformed cells by IIA may then reduce the pro-carcinogenic effects of radiation. However, when the

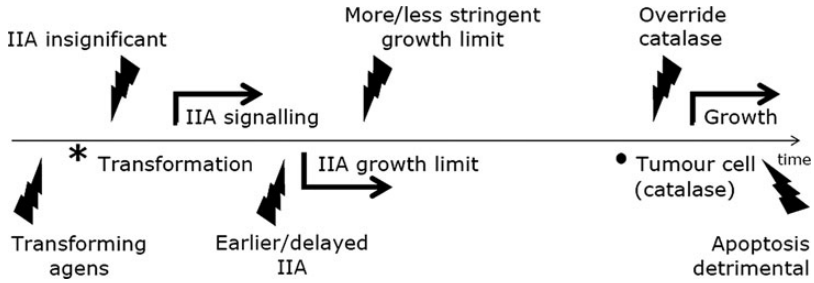

Figure 4. Radiation-induced modifications to the signalling that underlies the IIA may affect low-dose carcinogenesis differently, depending on the timing (see text for details).

$ONOO^-$ pathway in inter-culture mode dominates (Figure 2) or through complex consumption reactions between the two pathways[9], the radiation-induced enhancement of species release may reduce, rather than increase, the signalling efficiency. Radiation-induced modulation of IIA would be pro-carcinogenic, rather than protective, then.

The potential impact of radiation-induced modifications to IIA on low-dose carcinogenesis is schematically depicted in Figure 4. Radiation may affect the phenomenon differently depending on the exposure timing. If the cells are irradiated before the transformation took place, the well-known pro-transforming effects of radiation occur. Relatively shortly after transformation, when TGF-β signalling is already active but the density of transformed cells is still too low for apoptotic signalling to be effective, enhancement of signalling by radiation does not increase the $^{\bullet}OH$ yields sufficiently, and the phenomenon remains ineffective. Radiation exposure at a somewhat later stage, however, may shift the onset of effective signalling and induction of apoptosis to earlier or later times. Signalling modulation by radiation during the 'window of opportunity' when the transformed population growth has been halted may result in a (transiently) more or less stringent growth limit, enhancing or reducing the opportunity for other anti-carcinogenic mechanisms. Irradiation at later stages when cells exhibit catalase may result in $O_2^{-\bullet}$ production high enough so that its dismutation product, H_2O_2, overwhelms the catalase, and the signalling may get restored. Induction of apoptosis in tumour cells at relatively late stages may, however, be detrimental, as this may promote the growth of cancer stem cells and hence tumour progression[17].

In summary, IIA likely represents an important natural anti-carcinogenic mechanism. Low-dose radiation transiently modulates the underlying signalling processes. Depending on system parameters, these perturbations may act on the phenomenon in an anti- or pro-carcinogenic way.

ACKNOWLEDGEMENTS

Numerous discussions with Prof Georg Bauer (University of Freiburg, Germany), Dr Peter Jacob (Helmholtz Zentrum München, Germany), Prof Peter O'Neill, Drs Mark Hill and Luca Mariotti (University of Oxford, UK) are gratefully acknowledged.

FUNDING

The research leading to these results has received funding from the Seventh Framework Programme of the European Atomic Energy Community (Euratom) under grant agreements 249689 (DoReMi) and 269553 (EpiRadBio).

REFERENCES

1. Bechtel, W. and Bauer, G. *Catalase protects tumor cells from apoptosis induction by intercellular ROS signalling*. Anticancer Res. **29**, 4541–4557 (2009).
2. Hanahan, D. and Weinberg, R. A. *The hallmarks of cancer*. Cell **100**, 57–70 (2000).
3. Deichman, G. I. *Early phenotypic changes of in vitro transformed cells during in vivo progression: possible role of the host innate immunity*. Semin. Cancer Biol. **12**, 317–326 (2002).
4. Barcellos-Hoff, M. H. *It takes a tissue to make a tumor: epigenetics, cancer and the microenvironment*. J. Mammary Gland Biol. Neoplasia **6**, 213–221 (2001).
5. Bauer, G. *Elimination of transformed cells by normal cells: a novel concept for the control of carcinogenesis*. Histol. Histopathol. **11**, 237–255 (1996).
6. Bauer, G. *Low dose radiation and intercellular induction of apoptosis: potential implications for the control of oncogenesis*. Int. J. Radiat. Biol. **83**, 873–888 (2007).
7. Portess, D. I., Bauer, G., Hill, M. A. and O'Neill, P. *Low-dose irradiation of nontransformed cells stimulates the selective removal of precancerous cells via intercellular induction of apoptosis*. Cancer Res. **67**, 1246–1253 (2007).
8. Temme, J. and Bauer, G. *Low-dose gamma irradiation enhances superoxide anion production by nonirradiated cells through TGF-β1-dependent bystander signaling*. Radiat. Res. **179**, 422–432 (2013).
9. Bauer, G. *Low dose gamma irradiation enhances defined signalling components of intercellular reactive oxygen-mediated apoptosis induction*. J. Phys. Conf. Ser. **261**, 012001 (2011).
10. Kundrát, P., Bauer, G., Jacob, P. and Friedland, W. *Mechanistic modelling suggests that the size of preneoplastic lesions is limited by intercellular induction of apoptosis in oncogenically transformed cells*. Carcinogenesis **33**, 253–259 (2012).
11. Kundrát, P., Friedland, W. and Jacob, P. *Modelling of intercellular induction of apoptosis in oncogenic transformed cells and radiation effects on the phenomenon*. Radiat. Prot. Dosim. **143**, 549–553 (2011).
12. Ivanovas, B., Zerweck, A. and Bauer, G. *Selective and non-selective apoptosis induction in transformed and non-transformed fibroblasts by exogenous reactive oxygen and nitrogen species*. Anticancer Res. **22**, 841–856 (2002).
13. Herdener, M., Heigold, S., Saran, M. and Bauer, G. *Target cell-derived superoxide anions cause efficiency and selectivity of intercellular induction of apoptosis*. Free Radic. Biol. Med. **29**, 1260–1271 (2000).
14. Abdelrazzak, A. B., Stevens, D. L., Bauer, G., O'Neill, P. and Hill, M. A. *The role of radiation quality in the stimulation of intercellular induction of apoptosis in transformed cells at very low doses*. Radiat. Res. **176**, 346–355 (2011).
15. Portess, D. *An investigation into cellular signalling leading to the selective induction of apoptosis in transformed cells following radiation*. D.Phil. Thesis. University of Oxford (2007).
16. Saran, M. and Bors, W. *Signalling by O2−. and NO.: how far can either radical, or any specific reaction product, transmit a message under in vivo conditions?* Chem. Biol. Interact., **90**, 35–45 (1994).
17. Enderling, H., Anderson, A. R., Chaplain, M. A., Beheshti, A., Hlatky, L. and Hahnfeldt, P. *Paradoxical dependencies of tumor dormancy and progression on basic cell kinetics*. Cancer Res. **69**, 8814–8821 (2009).